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*Project Summary*

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# Increasing ARCAT® Test Sensitivity for Examination of Potable Waters

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The report summarized here describes the development of a method for concentrating coliphages from potable waters. The method is used in conjunction with a rapid coliphage analysis technique (ARCAT)* to detect 1 coliphage/100 ml of water sample. Seven concentration techniques were evaluated in preliminary feasibility studies, and three methods were selected for in-depth study based on recovery of coliphages, time required for availability of results, and ease of performing the techinque. Two of the three methods selected for in-depth studies were membrane filtration methods using positively charged and negatively charged filters. The third method involved amplification of coliphages by incubation to allow for a lytic burst to occur.

After in-depth studies and optimization of procedures, membrane filtration with the use of positively charged filters was selected as the concentration method of choice. Positively charged filters can be used successfully to concentrate coliphages present in potable waters at a level of 1 plaque forming unit (pfu)/100 ml of water sample using the ARCAT procedure. The ARCAT amplification technique can be used to obtain qualitative indication of the presence of coliphage when coliphage numbers are expected to be < 5/100 ml and can be run in parallel with the ARCAT/concentration procedure. Test results are available in 6.5 hours at an approximate cost of $4.12 per test. ARCAT results can be used to estimate the number of fecal and total coliforms in the water sample or may be used independently as an indicator of water quality.

Examination of the effects of physical treatment of water indicated that settling and filtration are effective means for reducing levels of coliphages and total and fecal coliforms in natural waters. Coliphages appeared to be less efficiently removed by these processes than total and fecal coliforms.

Disinfection studies indicated that chlorine dioxide was effective as a viricide and bactericide. At ozone levels achieved in this study, reductions in coliphages and coliforms were not consistent. Ozone disinfection effectiveness was both concentration and exposure-time dependent.

*This Project Summary was developed by EPA's Municipal Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).*

*Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## Introduction

Waterborne diseases can be spread rapidly by bacterial and/or viral contaminants in any waters used for human consumption. Community water supplies are treated to remove or reduce these contaminants by such methods as settling, filtration, and disinfection. Although the most probable number (MPN) and the membrane filtration (MF) procedures for detecting total coliform bacteria (indicators of fecal contamination) are standard tests for determining the quality of treated water, the minimum time for results from these procedures is 24 ± 2 hours. A rapid test to evaluate the sanitary quality of water could be used to decide whether or not additional treatment, such as booster chlorination in the system, would be necessary.

Under contracts with the U.S. Department of the Army, Atlantic Research Corporation (ARC) developed a rapid test to determine the number of bacteriophages of a given bacterial species in a water sample within 4 to 6 hours. The detection of coliphages was of particular interest, and a quantitative relationship between coliform bacteria and their coliphages (infective for *Escherichia coli* C) was established.

Field tests of the coliphage method, ARCAT, were conducted in 1977 and 1978. The quantitative relationship between coliphages and coliforms established in the earlier studies was confirmed and shown to be valid for natural waters sampled throughout the United States.

Under a National Science Foundation Grant, the test procedure was simplified and conditions for coliphage replication and plaque formation were optimized. The sensitivity of ARCAT following these modifications was 5 coliphages/100 ml of water (based on plating four 5-ml aliquots of the water sample). At this sensitivity, the method was not suitable for determination of water quality in the potable water range. The study reported here focused on increasing the sensitivity of ARCAT to the level of 1 coliphage/100 ml.

## Results and Discussion

The concentration techniques evaluated to increase the sensitivity of ARCAT to detect 1 coliphage/100 ml of water sample are listed below:

- Concentration by filtration through positively charged filters
- Concentration by filtration through negatively charged filters
- Osmotic filtration (dialysis)
- Controlled lytic bursts
- Adsorption on inorganic salts
- Polymer two-phase separation
- Magnetic filtration
- Electrophoresis

From Table 1, the percent of coliphage recoveries achieved by the concentration techniques, the relative complexity of the method, and the time factor increase over the ARCAT test alone can be compared.

Three methods were selected for in-depth study based on recovery of coliphages, time required to obtain test results, and ease of performing the technique. Concentration using positively charged and negatively charged filters yielded good coliphage recoveries and added 0.5 hour and 1.5 hours, respectively, to the time required for ARCAT. A third method, amplification of coliphages by incubation to allow a lytic burst to occur, increased the time required for availability of test results by 70 minutes.

Concentration of coliphages with positively and negatively charged filter methods was initially evaluated with the use of sterilized water samples seeded with known concentrations of stock coliphages. Both filter types demonstrated good adsorption, and coliphage recoveries of greater than 70% were generally obtained. These filtration techniques were optimized with respect to coliphage adsorption and elution conditions. Because of occasional clogging of the negatively charged filters and the additional hour required to concentrate the sample using the negatively charged filter, the positively charged filter was selected as the filtration method of choice. The AMF Cuno Zeta Plus 60S positively charged filter consistently had the best coliphage adsorption (80% to 100%) and recoveries (≥90%). The filter performed best when the water sample was adjusted to pH 6.0 before filtration. Recoveries of the coliphages from the filter were consistently high when 3% Trypticase Soy Broth adjusted to pH 8.5 was used as the eluent.

Concentration of coliphages from natural waters using the Zeta Plus 60S filter was evaluated. Coliphage recoveries from the positively charged filter were compared with coliphages detected by plating 100 ml of the water sample in the ARCAT procedure. Table 2 shows coliphage recoveries from natural water samples; percentages recovered were more variable than those obtained using samples spiked with T2 and Ø174 coliphages (93% to 100%). The types and densities of coliphages present in the water samples were a factor in the variable recoveries. A severe drought during much of the

***Table 1.*** *Comparison of Concentration Methods*

| *Concentration Technique* | *Additional [a] Time Required* | *% Coliphage Recovered* | *Complexity of Technique [b]* |
|---|---|---|---|
| *Positive filters* | | | |
| *50S* | *30 min* | *13-73* | *2* |
| *70C* | *40 min* | *27-65* | *2* |
| *1MDS* | *15 min* | *42-108* | *2* |
| *Negative filters* | | | |
| *Cox 2 µm* | *15 min* | *3-47* | *3* |
| *Cox 0.45 µm* | *30-60 min* | *0-125* | *3* |
| *Bio-Rad 0.2 µm* | *> 2 hr* | *0-71* | *3* |
| *Osmotic filtration* | *8 hr [c]* | *0-150* | *2* |
| *Lytic burst* | *1 hr* | *[d]* | *2* |
| *Adsorption on inorganic salts* | *2 hr* | *0-9* | *3* |
| *Polymer two-phase separation* | *18-24 hr* | *100* | *4* |
| *Magnetic filtration* | *1 hr* | *0-37.5* | *4* |

*[a] Time in addition to that required for ARCAT. (Includes the set-up and performance of the concentration techniques.)*

*[b] Compared with ARCAT at complexity of 1. Complexity increased by additional manipulation of samples and materials preparation or increased length of time needed for processing.*

*[c] At least 8 hours were required to achieve a 2-fold concentration of 100 ml.*

*[d] This technique involves amplification of bacteriophages present, not recovery by concentration.*



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*Table 2.* *Correlation of Coliphage Results from ARCAT and Zeta Plus 60S Concentration/ARCAT Methods.*

| Date | Sample | Volume Filtered (ml) | Coliphage pfu[a] | | Coliphage % Recovered[d] |
|---|---|---|---|---|---|
| | | | ARCAT[b] | Eluate[c] | |
| 8/20 | Occoquan raw | 750 | 3 | 7 | 31.1 |
| 8/26 | Accotink | 500 | 6 | 39 | 130.0 |
| | Holmes run | 500 | 1 | 12 | 240.0 |
| 8/27 | Accotink | 500 | 4 | 19 | 95.0 |
| 8/28 | Potomac raw | 500 | 60 | 337 | 112.0 |
| 8/29 | Accotink | 500 | 6 | 16 | 53.3 |
| 9/02 | Cherokee | 500 | 8 | 58 | 145.0 |
| | Cherokee | 100 | 8 | 15 | 187.5 |
| 9/04 | Potomac raw | 500 | 41 | 143 | 69.8 |
| | Potomac raw | 100 | 41 | 22 | 53.6 |
| | Potomac settled | 1000 | 14 | 29 | 20.7 |
| 9/08 | Cherokee | 500 | 3 | 3 | 20.0 |
| 9/09 | Potomac raw | 100 | 27 | 26 | 96.3 |
| | Potomac raw | 500 | 27 | 111 | 82.2 |
| | Potomac raw | 1000 | 27 | 162 | 60.0 |
| | Potomac settled | 100 | 8 | 1 | 12.5 |
| | Potomac settled | 500 | 8 | 15 | 37.5 |
| | Potomac settled | 1000 | 8 | 141 | 176.3 |
| 9/15 | Potomac raw | 100 | 21 | 77 | 366.7 |
| | Potomac raw | 500 | 21 | 258 | 245.7 |
| | Potomac raw | 1000 | 21 | 335 | 159.9 |
| 9/15 | Potomac filtered | 500 | 4 | 22 | 110.0 |
| 9/16 | Potomac raw | 100 | 15 | 10 | 66.7 |
| | Potomac raw | 100 | 15 | 15* | 100.0 |
| | Potomac settled | 100 | 2 | 1 | 50.0 |
| | Potomac settled | 100 | 2 | 1* | 50.0 |
| 9/17 | Potomac raw | 100 | 15 | 21 | 140.0 |
| | Potomac raw | 100 | 15 | 10* | 66.7 |
| | Potomac settled | 100 | 5 | 5 | 100.0 |
| | Potomac settled | 100 | 5 | 8* | 160.0 |
| 9/18 | Potomac raw | 100 | 807 | 620 | 76.8 |
| | Potomac raw | 100 | 907 | 391* | 48.4 |
| | Potomac settled | 100 | 295 | 295 | 100.0 |
| | Potomac settled | 100 | 295 | 159* | 53.9 |
| 9/19 | Potomac raw | 100 | 241 | 239 | 99.2 |
| | Potomac settled | 100 | 72 | 19 | 26.4 |
| | Potomac settled | 100 | 72 | 9* | 12.5 |
| 9/22 | Potomac settled | 100 | 3 | 3 | 100.0 |
| | Potomac filtered | 100 | 1 | 2 | 200.0 |
| 9/23 | Potomac raw | 200 | 9 | 40 | 222.2 |
| | Potomac settled | 200 | 5 | 6 | 120.0 |

[a] *Plaque forming units.*
[b] *Coliphage pfu/100 ml determined by ARCAT.*
[c] *Coliphage pfu in eluate. "*" indicates 10 ml eluate volume plated; all others were 20 ml.*
[d] $\frac{\text{Eluate pfu}}{\text{ARCAT pfu (multiplication factor)}} \times 100.$
*Multiplication factor is number of 100 ml volumes equal to original volume filtered for concentration.*

study period resulted in unusually low or variable coliphage occurrences compared to previous nondrought periods.

Least squares linear regression analysis of the data shown in Table 2 was used to determine how well the Zeta Plus concentration method predicted the number of coliphages originally present in the natural water sample. The regression (Figure 1) was highly significant with a coefficient of determination of 0.8516, indicating that more than 85% of the variation in the coliphage determination was related to the initial concentration of coliphages filtered. The accuracy of the Zeta Plus concentration method in predicting coliphages present in the original water sample is illustrated in Table 3; calculations were made using the regression line equation: $Y_{pred} = 1.2853 X - 3.61$.

Detection of low level (1 to 5 pfu/100 ml) coliphage contamination in water samples using ARCAT without concentration was done by amplifying the coliphage(s) present in the sample. Nutrient addition and addition of the *E. coli* C host to a final concentration of 1 x 108 cells/ml in the amplification flask were required. Incubation of the amplification sample (100 ml, 200 ml) for 70 minutes at 35°C before plating in ARCAT provided optimum amplification of coliphages for which *E. coli* C is a suitable host. The amplification technique was most valuable when used in conjunction with the standard ARCAT procedure for waters expected to contain less than 5 coliphages/100 ml (the detection limit for the standard ARCAT procedure). If coliphages were not found by the standard ARCAT procedure, the amplification technique allowed the user to determine if any coliphages were present. Thus, the amplification technique yielded only qualitative results at the 1 to 5 coliphage/100 ml level.

Physical treatment of water (settling, filtration) reduced coliphages, total coliforms, and fecal coliforms by 90% to 100%. In a brief study of the effects of ultraviolet light, chlorine dioxide, and ozone disinfectants, coliphages appeared to be as sensitive to ultraviolet light as were total coliforms and fecal coliforms. At a concentration of 5 mg/L, chlorine dioxide was effective as a viricide and in reducing fecal and total coliforms. Reduction of coliphages, total coliforms, and fecal coliforms by exposure to ozone was time and ozone-concentration dependent. Bacteriophage and coliform reductions were not consistent at the ozone concentrations achieved during this study.

The ARCAT concentration procedure for detecting 1 coliphage/100 ml of water is given below:

- Adjust water sample (100 to 1000 ml) to pH 6.0 with 0.1 M or 1.0 M HCl
- Filter water sample at pH 6.0 through the Zeta Plus 60S filter with vacuum
- Remove flask containing the filtrate and replace with a sterile flask



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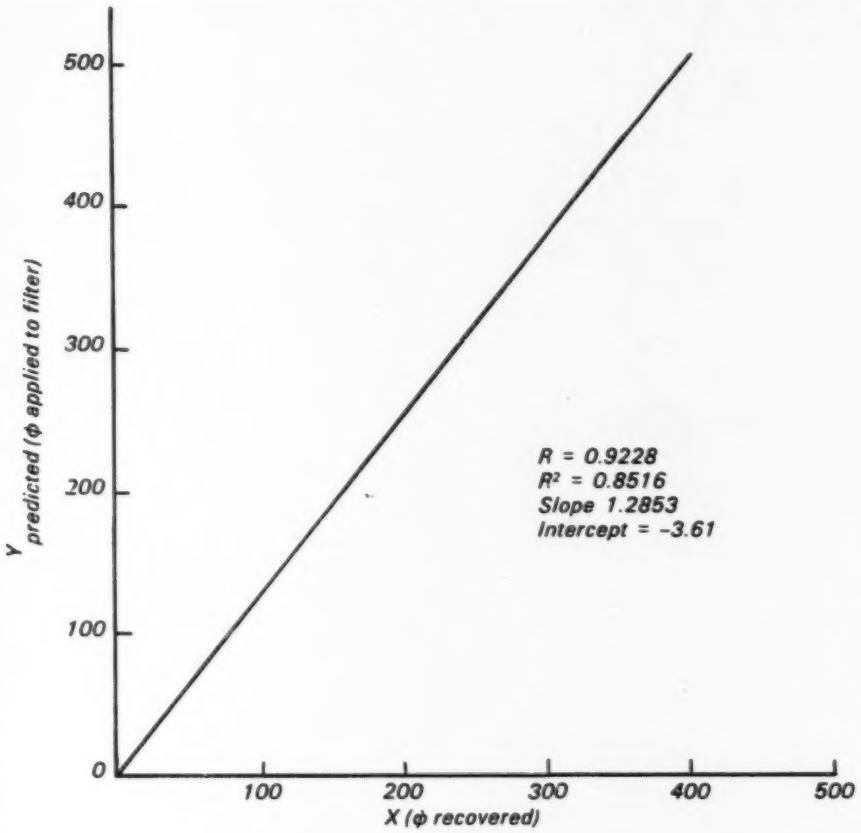


*Figure 1.* *Least squares regression line of coliphages recovered versus coliphages filtered.*

*Table 3.* *Predicted Coliphage Versus Coliphage Applied.*

| Coliphage (pfu) Detected by ARCAT Concentration (X) | Coliphage (pfu) Predicted [a] (Y) | Coliphage (pfu) Applied [b] |
|---|---|---|
| 1 | — | 2, 2 |
| 2 | — | 1 |
| 3 | 0.23 | 3 |
| 5 | 2.8 | 5 |
| 6 | 4.1 | 10 |
| 8 | 6.6 | 5 |
| 10 | 9.2 | 15, 15 |
| 12 | 11.75 | 5 |
| 15 | 15.59 | 8, 15 |
| 16 | 16.87 | 30 |
| 21 | 23.3 | 15 |
| 22 | 24.6 | 20, 41 |

[a] *The sensitivity of the method is ≥ 3 pfu applied to the filter.*
[b] *Coliphage applied was determined by plating 100 ml in the regular ARCAT procedure.*

- Apply 10 ml of trypticase soy broth at pH 8.5 to the filter, and allow a contact time of 5 minutes before vacuum is applied
- Apply a second 10 ml aliquot of TSB at pH 8.5 to the filter
- Wash the filter with three 1-ml aliquots of sterile distilled water
- Divide the total eluate (20 ml) between four tubes each containing 5.5 ml of molten modified nutrient agar
- Add 1 ml of *E. coli* C host to each tube
- Mix the contents of each tube and decant into a sterile Petri dish; allow agar to solidify and incubate at 35°C for 6 hours
- Enumerate plaques at 6 hours

## Results

In summary, the use of the Zeta Plus 60S positively charged filter to concentrate low levels of coliphages in a water sample provided the increase in ARCAT test sensitivity needed for routine examination of potable waters for coliphages. Test results were obtained in 6.5 hours at an approximate cost of $4.12 per test. The ARCAT results can be used to estimate the number of fecal and total coliforms in the water sample or may be used independently as an indicator of water quality.

## Recommendations

1. ARCAT sensitivity has been increased using the Zeta Plus 60S concentration method for determination of water quality in the potable water range. This method should be field tested in water quality laboratories in various geographical areas in the United States. Laboratories selected for testing should examine a wide variety of water samples, treatment processes, and testing expertise. Each laboratory should evaluate at least 50 samples during a 1-year period. Data for each sample should include: 1) water pH and turbidity, 2) sample volume, 3) number of coliphages detected, 4) number of fecal and total coliforms detected, and 5) virus data, if available. Data should be collected and evaluated at a central laboratory.

2. Amplification of coliphages in natural water samples can detect low level contamination but does not allow for quantification of the actual coliphages present in the original sample. Further study of coliphage morphology, plaque size, burst size, and the significance of



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the presence of any one coliphage type in a water sample is needed. The types of coliphages present in natural waters must first be isolated and identified, however. Correlation of specific coliphages with epidemiological information should yield a predictive test for waterbone disease outbreak.

3. Coliphages survive physical water treatment processes as well as or perhaps better than coliform bacteria. A study to correlate enteric virus and coliphage survival through water treatment processes is recommended. One or two enteric viruses should be selected for initial studies to compare survival of the viruses with that of the coliphages following water treatment processes under controlled conditions. Survival of the viruses and coliphages should then be evaluated under actual treatment conditions.

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***Donald J. Reasoner** is the EPA Project Officer (see below).*
*The complete report, entitled "Increasing ARCAT® Test Sensitivity for Examination of Potable Waters," (Order No. PB 82-196 163; Cost: $12.00, subject to change) will be available only from:*
*National Technical Information Service*
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